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Abstract of the PhD thesis entitled:

A computational model of molecular mechanism behind the half-the-sites activity of thymidylate synthase

The title in Polish:

Model obliczeniowy molekularnego mechanizmu aktywności typu half-the-sites syntazy tymidylanowej

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Proteins have been the subject of many experimental and theoretical studies that covered various aspects of their structure and function, e.g., structure prediction (basing on amino acid sequence), substrate recognition, allostery, protein folding/unfolding, RNA-binding, conformational changes in proteins.

The studies conducted by Wojciech Rode and his group from Nencki Institute of Experimental Biology Polish Academy of Sciences regarding various aspects of biosynthesis of thymidylate, such as its enzymology, regulation and inhibition, with particular interest in thymidylate synthase – searching for new inhibitors/drugs, drug resistance, specificity and mechanism of action, as well as mechanisms of enzyme inhibition and its specificity – became an inspiration to undertake theoretical studies in this field. The subject of the presented dissertation has been inspired by numerous discussions on the molecular basis of functioning of this enzyme.

One of the alluring and significant proteins is thymidylate synthase (TS), an enzyme catalyzing reductive methylation of deoxyuridine monophosphate (dUMP) using 5,10-methylene-5,6,7,8-tetrahydrofolate as a cofactor, and yielding deoxythymidine monophosphate (dTMP) and dihydrofolate. Thus, it plays an enormous role in DNA replication providing a precursor of one of the nucleotides (dTTP). And since it is the sole source of dTMP in a cell, it is an important target enzyme in anticancer chemotherapy, as well as in antiviral,

antiprotozoal, and antifungal therapies. Its structure, catalytic role, and other aspects of its function are presented in chapter 2.

Lately, there has been posed a plausible hypothesis that thymidylate synthase is a half-the-sites activity enzyme, what implies a "communication" of its both subunits while binding a substrate and a cofactor in the catalytic reaction. It is a fascinating, yet not extensively investigated problem. This study is an attempt to address this phenomenon from two different perspectives.

In the first approach the low-frequency normal modes of ligandless thymidylate synthase were investigated. These low-frequency modes relate to collective motions of segments in a protein and are of great biological importance, e.g., they contribute to opening and closing of the active sites. This involves a complex motion of many different parts of the enzyme: the less localized the normal mode, the more portions of the protein it entails.

With the use of the web interface to the Elastic Network Model, ElNémo, we have identified several symmetric and asymmetric normal modes. Their analysis demonstrated the differences in the behaviour of each of the two active sites, as well as a nonequivalence of the two subunits of TS dimer, suggesting a possible pathway of communication between them. Seven symmetric and nine asymmetric normal modes display SASA (*solvent-accessible surface area*) changes in both active sites of the dimer. SASA values are usually greater in one subunit and are accompanied by synchronized changes in the values of chosen distance monitors.

The asymmetric modes are the most common amongst the 25 lowest-frequency normal modes for the enzyme. While performing them, the protein facilitates the access to one active site, hindering simultaneously the access to the other one. The most engaged sections of TS are the loops lining the entrance to the active site, as well as helix K – they have their part in almost every normal mode and happen to be the three segments that contribute to active site closure upon ligands binding. One of these loops includes mammalian specific insertion, containing S114, and can perform both hinge and shear motion. The other loop is the N-terminal one which serves as a lid to the active site pocket and provides with amino acids that sense the presence of a substrate on one hand and take part in the intersubunit communication on the other hand.

Based on the literature data that regards relaying structural changes from one active site to the other through the interface and connected with it negative cooperativity in ligand binding, our studies trace and confirm the differences in the proposed packing interactions at the edges of the β-sheet as a possible pathway communicating the two active sites. Along with another observation that while executing normal modes, especially the asymmetric ones, thymidylate synthase hampers the access to one active center and makes it easy to the other, which represents an important dynamic feature of the enzyme, these probably account for the half-the-sites activity.

This nonequivalence in the behaviour of the two subunits and their active sites of the studied protein was also recognized in molecular dynamics simulation studies in denaturing environment of 8-molar urea or 6-molar guanidinium chloride. These studies were conducted for two versions of thymidylate synthase dimer (AB and CD) with different electron densities.

In such conditions, thymidylate synthase kept a substantial portion of ordered structure, the fact that is acknowledged by the literature data on research of proteins submitted to strongly denaturing conditions. But at the same time, the three segments of TS that contribute to active site closure upon ligands binding to it — two loops and helix K, turned out to be the most mobile part of the enzyme. While in urea, they were frolicking during the whole simulation time, however this occurred differently in both subunits (no matter if AB or CD dimer was take into account). The displacement was always greater in A or C monomer as to partially destruct the tertiary structure, however greater changes in the secondary structure were found in B or D subunit.

Also, the very beginning of unfolding of thymidylate synthase dimer was observed. The chosen conditions (8 M urea or 6 M guanidinium chloride and temperature of 310 K) allowed for detecting only early changes in the protein structure, but are the first that model the influence of solely the denaturant, and not the temperature, on protein. The observed unfolding was proved by increasing RMSD and solvent-accesible surface area values, and proceeded unequally with respect to dimer subunits. The changes were always greater in one subunit and occurred unevenly. This was followed by the changes in the arrangement of amino acids involved in active site formation, as some of them were found far from their original location. This is an argument in favor of the hypothesis that TS is a half-the-sites activity enzyme.

Abundant literature data on the topic of unfolding of proteins reveals that denaturation of proteins proceeds much more easily in GuHCl than in urea, even when smaller concentrations of the former are used. However, our results demonstrate something opposite. Guanidinium chloride brought about only small response in the protein structure, while urea caused the protein to lose locally its tertiary structure, thus pointing out that urea is more potent denaturant than GuHCl, at least in the applied concentrations (8 M for urea and 6 M for GuHCl). However, the elucidation of the molecular basis of the experimentally established easier denaturation of proteins in GuHCl than in urea requires further MD simulations, including the use of wider spectrum of concentration of denaturing agents, prolonged simulation times, or investigating the temperature effect.

The dissertation was written in English. The results of the performed studies were published in two original scientific articles. They were also presented at several domestic and international conferences, both in the form of oral presentations and posters.